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Investigating the Parasitism of Southern California Bean Clams (*Donax gouldii*) by the Trematode *Postmonorchis donacis*

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Abstract.—The bean clam, *Donax gouldii*, is an intermediate host of the monorchid trematode *Postmonorchis donacis*. Bean clams were collected from nine locations in San Diego County, CA, and siphons and mantle edges examined. Two hypotheses were tested: (1) parasitism increases with valve length, and (2) female clams have more parasites than males. A positive relationship was found between clam length and parasitism at all locations; there was no significant difference ($\alpha = 0.05$) in male and female parasitism rates. Spatial variation on a kilometer scale was observed in trematode infestation rate and intensity.

The study of parasitism can provide critical insights into the natural histories of many organisms, and the investigation of parasite/host interactions is vital to understanding interactions among species, communities, and ecosystems. Young (1953) reports finding a new species of monorchid trematode, *Postmonorchis donacis*, and describes the species and some of its life history. *Postmonorchis donacis* uses the marine bivalve *Donax gouldii*, the bean clam, as a second intermediate host, primarily for the metacercarial stage of the trematode. *Donax gouldii* was studied intensively for 17 years by Coe (1955), who found that bean clams have extreme fluctuations in population densities. During the years 1949 to 1952 at the Scripps Coastal Reserve, *D. gouldii* had a peak population density of about 20,000 clams/m^2, with a density of less than 1 clam/m^2 in the years immediately preceding and following that surge in numbers (Coe 1955); no certain explanation was found for the phenomenon.

D. gouldii is a veneroid bivalve mollusk that ranges from Point Conception, California to Southern Baja California, Mexico and lives on open coast sandy beaches in a fixed intertidal position, unlike other *Donax* species (Irwin 1973; Ellers 1995). They live to a maximum age of three years and reach a length of approximately 25mm; their triangular shells are generally colored buff and yellow and have low radial ribs (Haderlie and Abbott 1980). The primary consumers of *D. gouldii* are rays, spotfin croakers, surfperches, and sea gulls (Love 1991). Populations of *D. gouldii* can be highly variable, with population resurgences occurring every 2 to 14 years (Coe 1953). *Donax gouldii* are broadcast spawners whose females mature after one year and produce approximately 50,000 eggs at each spawning, which may occur several times in a year (Haderlie and Abbott 1980).

Postmonorchis donacis is a monorchid trematode that uses at least two hosts during its life cycle. The adult trematode uses the hind gut of nearshore teleost fish and elasmobranches as a definitive host and location for sexual reproduction. Sexually produced eggs settle and hatch in the primary intermediate host, which Young (1953) hypothesized to be a copepod. The primary intermediate host is castrated by the sporocyst life stage of the parasite, which asexually reproduces to form cercariae with pigment spots, ventral suckers, and tails formed of overlapping scales. The cercariae are free swimming and mature into metacercariae, encysted larval trematodes lacking a tail, after entering their second intermediate host, *D. gouldii*; it has been hypothesized that *D. gouldii* become infested through the consumption of the small, parasitized copepods (Young 1953). The metacercarial cysts are found in the siphons and along the mantle edges of *D. gouldii*. When metacercariae are ingested by their definitive host, they excyst and mature in the fish's hind gut (Young 1953).

Young (1953) was unable to obtain parasitized clams by exposing them to trematode eggs and hypothesized a three-host life history model for *P. donacis*. However, other, related species of trematode within the family Monorchiidae (Subclass Digenea) use only one intermediate host: a single species of clam for both sporocyst and metacercarial life stages (DeMartini and Pratt 1964). As yet, little work has been done on the factors contributing to the rates of infestation of *P. donacis* in *D. gouldii*. The trematodes have never before been quantified with respect to the size, sex, or population density of their clam hosts, nor have they been studied across a broad spatial scale.

The purpose of this study was to examine trematode infestation rates in *D. gouldii* and possible factors contributing to the infestation levels of *P. donacis*. To quantify the factors determining *P. donacis* infestation rates in *D. gouldii*, we hypothesize that (1) with size as a proxy for exposure time, the number of metacercariae per clam will increase with valve length, and (2) because female bivalves are often larger than conspecific males, parasites will be more numerous in female clams.

Materials and Methods

Donax gouldii were collected from San Diego County, CA, between January 21, 2009, and May 6, 2009, from tidal heights ranging from −0.3m MLLW to +0.3m MLLW. The initial goal of this study was to develop a time series of trematode infestation. Clams were sampled from the Scripps Coastal Reserve (SCR) on January 21 (n = 15), January 28 (n = 10), February 2 (n = 22), February 24 (n = 21), April 2 (n = 20), and May 6 (n = 31), 2009. Interesting infestation patterns led us to examine *D. gouldii* populations over a larger spatial scale. Clams were collected from sites south of SCR on April 6, 2009 – Mission Beach, CA (MB) (n = 20), the northern most end of the Silver Strand, CA (NSS) (n = 24), and just north of the Tijuana River Estuary (TRE) (n = 21) – and from sites north of SCR on April 20, 2009 – Oceanside, CA (OS) (n = 20), Carlsbad, CA (CB) (n = 17), the edge of Carlsbad and Leucadia, CA (C/L) (n = 21), Solana Beach, CA (SB) (n = 22), and Torrey Pines State Beach (TP) (n = 22) (Figure 1).

Donax gouldii were dissected fresh, after refrigeration for one to three days, or after freezing for up to one month. If not dissected fresh, the clams were refrigerated or frozen after having been stored in filtered sea water. Each clam was opened with the use of a scalpel, and the viscera removed from the valves. Valve length was measured anterior to posterior in millimeters, using calipers with accuracy to 0.05mm.

The gonads were inspected under a dissecting scope for the presence of *Postmonorchis donacis* and to determine gender (if mature). Siphons and mantle edges were slide mounted and examined under transmitted light on a compound microscope to count metacercariae present (1/21/09 collection only counted up to 100). Metacercarial cysts were positively identified using Young's 1953 species description and figures. One-tailed, two sample *t*-tests were used to examine differences in male and female valve lengths and metacercarial counts. Cyst counts between locations were analyzed using Tukey's honest significance test, which compares location means and groups sites by similarity.

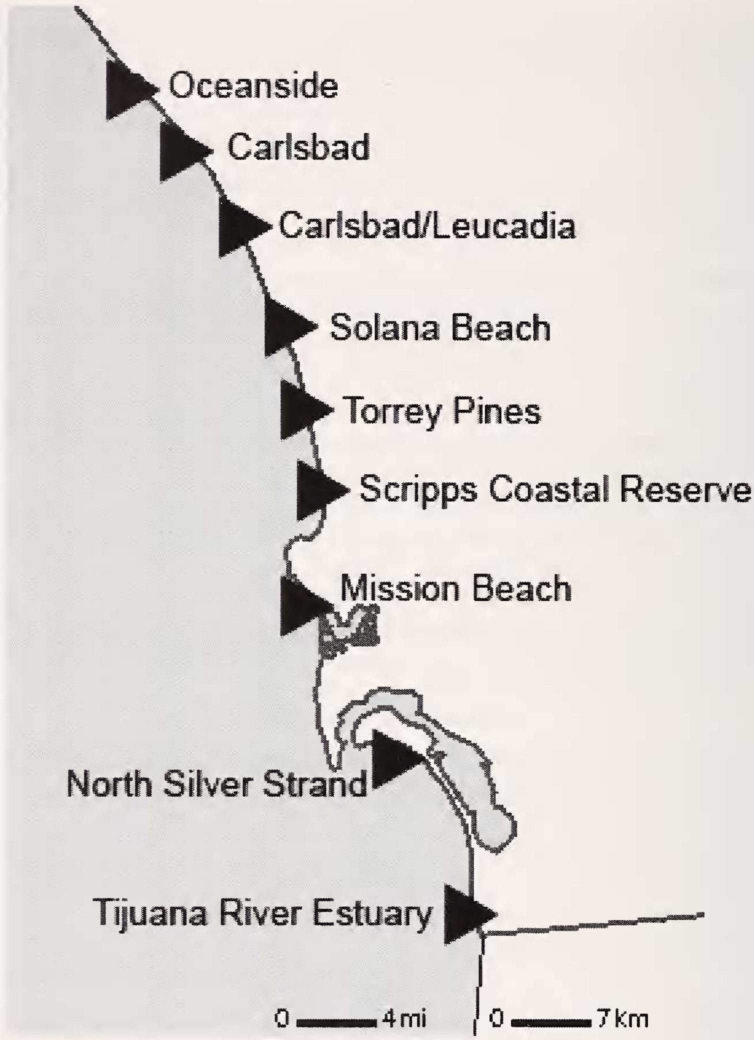

Fig. 1. Map of approximately 32° north, 117° west, showing the locations of the nine collection sites. Listed north to south, the locations are Oceanside (OS), Carlsbad (CB), Carlsbad/ Leucadia (C/L), Solana Beach (SB), Torrey Pines (TP), Scripps Coastal Reserve (SCR), Mission Beach (MB), North Silver Strand (NSS), and Tijuana River Estuary (TRE).

Population density measurements for *D. gouldii* were taken at all locations during the lower low tides of a spring tidal cycle: OS, CB, and C/L on May 26; MB, NSS, and TRE on May 27, 2009; and SB, TP, and SCR on June 4, 2009. At each location, density was measured in at least four quadrats (50cm by 50cm), with two quadrats haphazardly placed at two or three tidal heights; densities were measured at or near the −0.3m, 0.0m, and +0.3m mean low low water (MLLW) tide lines, which were determined by level scope and stadia rod. Sand was removed from each quadrat to an approximate depth of 15cm and sieved using a 2mm mesh; all *D. gouldii* recovered were collected for later analysis. Average population densities at low, mid, and high tidal heights were calculated by location.

Table 1. Metacercariae and valve length means calculated by site. Outliers were removed from NSS and TP, with 1221 and 412 metacercariae, respectively. Note that density measurements and clam collections were made on different days.

Location	OS	CB	C/L	SB	TP	SCR	MB	NSS	TRE
Mean metacercariae	6.69	6.83	3.32	16.03	20.88	118.15	2.9	131.24	0.53
Mean length	10.76	12.26	12.42	11.76	11.66	11.94	10.14	13.92	10.07
Density/m^2	3.4	5	0	12	22.67	2	0	1.33	0.8
R^2 significance:									
two-tailed p-values	<0.0001	0.0012	0.0020	<0.0001	<0.0001	<0.0001	0.0009	0.0004	0.0094
# *D. gouldii* sampled	38	29	28	34	56	106	20	29	30
% with metacercariae	87%	64%	68%	71%	71%	99%	60%	100%	30%

Results

A total of 378 *Donax gouldii* received absolute counts of metacercariae. The metacercariae were observed to concentrate at the base of the inhalant siphon of the clam host. Of the 378 clams, 298 (79%) had at least one *Postmonorchis donacis* metacercaria; however, strong spatial variability was observed. Silver Strand (NSS) had the highest infestation rate at 100% (n = 29) followed by SCR with 99% infested (n = 106), while TRE had the lowest at 30% (n = 30) (Table 1). Metacercaria counts ranged from 0 to 1,221, with an overall mean count of 50.32. Silver Strand and SCR had the highest mean number of metacercariae per clam at 131 and 118, respectively, while TRE had the lowest at 0.53 (Table 1; for median and data range see Figure 2).

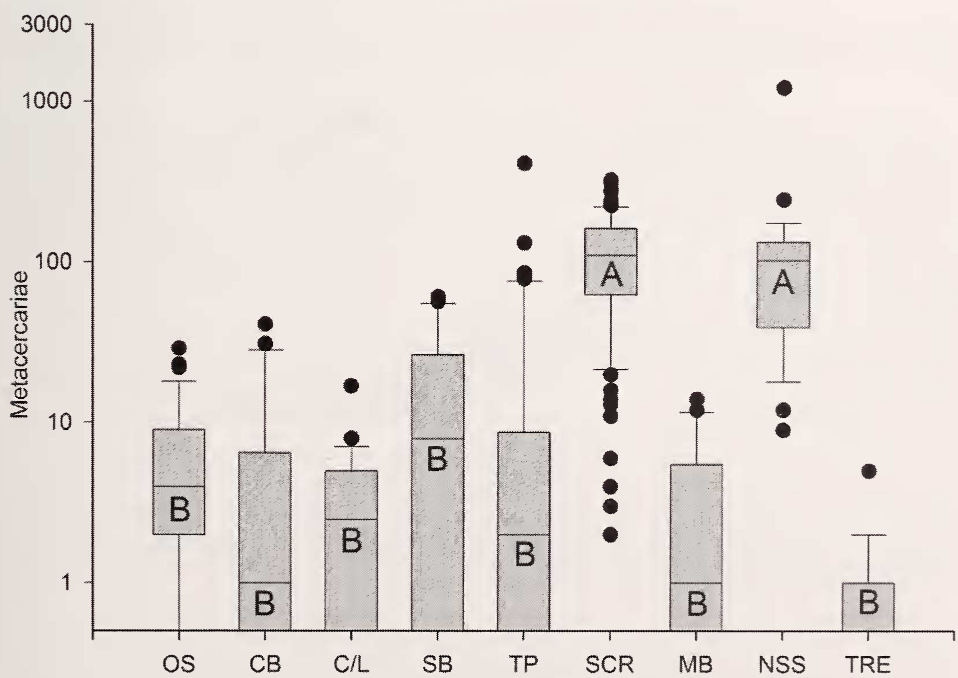

Fig. 2. Box plot of $\log_{10}$ metacercaria counts per *D. gouldii* for all study locations. The center line is the median, and the box extends one standard deviation, with whiskers extending to two standard deviations. Outliers are plotted as circles. Results of Tukey's honest significant difference are shown in lettered groups A and B.

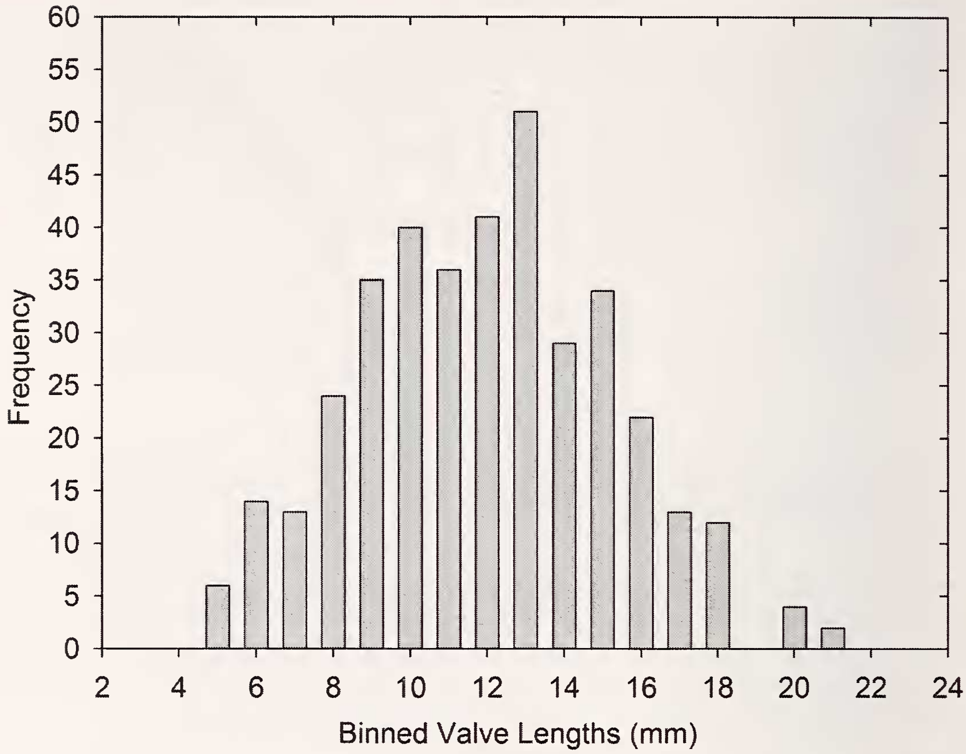

Fig. 3. Frequency of each binned valve length over all locations. The average valve length is 11.40mm. The data have a median of 11.50mm and a mode of 8.95mm and show a rather normal distribution.

Analysis of the nine locations using Tukey's honest significance test resulted in two groups: NSS and SCR in one and the other seven sites in another (Figure 2). Sporocysts and/or cercariae were found in three of the 386 examined *D. gouldii*: two collected from SCR and one from NSS, the two locations with the highest mean metacercaria counts; the three individuals infested with sporocysts each had metacercarial counts greater than 100. Over all locations, valve length averaged 11.42mm with a range of 4.15 mm to 20.80mm (Figure 3). Silver Strand had the largest average shell length at 13.92 mm, while TRE had the smallest at 10.07mm (Table 1). A positive relationship between valve length and number of metacercariae was found at all locations (Table 1, Figure 4). Clams collected from TRE had the weakest relationship between shell length and metacercariae, the lowest average metacercariae load, and the smallest average shell length (Table 1). The strongest exponential relationship was found at TP, with an R^2 of 0.716. Trematodes were absent or infrequent in the smallest individuals (under 7mm; n = 32). Only 25% of those small clams had metacercariae, with a maximum cyst count of 4. No individuals smaller than 5.60mm (n = 11) contained metacercariae.

The relationship between gender and number of metacercariae was examined. Male clams (n = 173) had an average of 48.98 metacercariae, females (n = 191) an average of 52.77, and clams whose gonads were undifferentiated (n = 14) an average of 31.86. Differences in male and female counts of metacercariae were not significant ($\alpha = 0.05$). Females had a mean size of 11.26mm, while males had a mean of 11.76mm; clams with undifferentiated gonads had a mean of 9.01mm. Though male clams were larger on

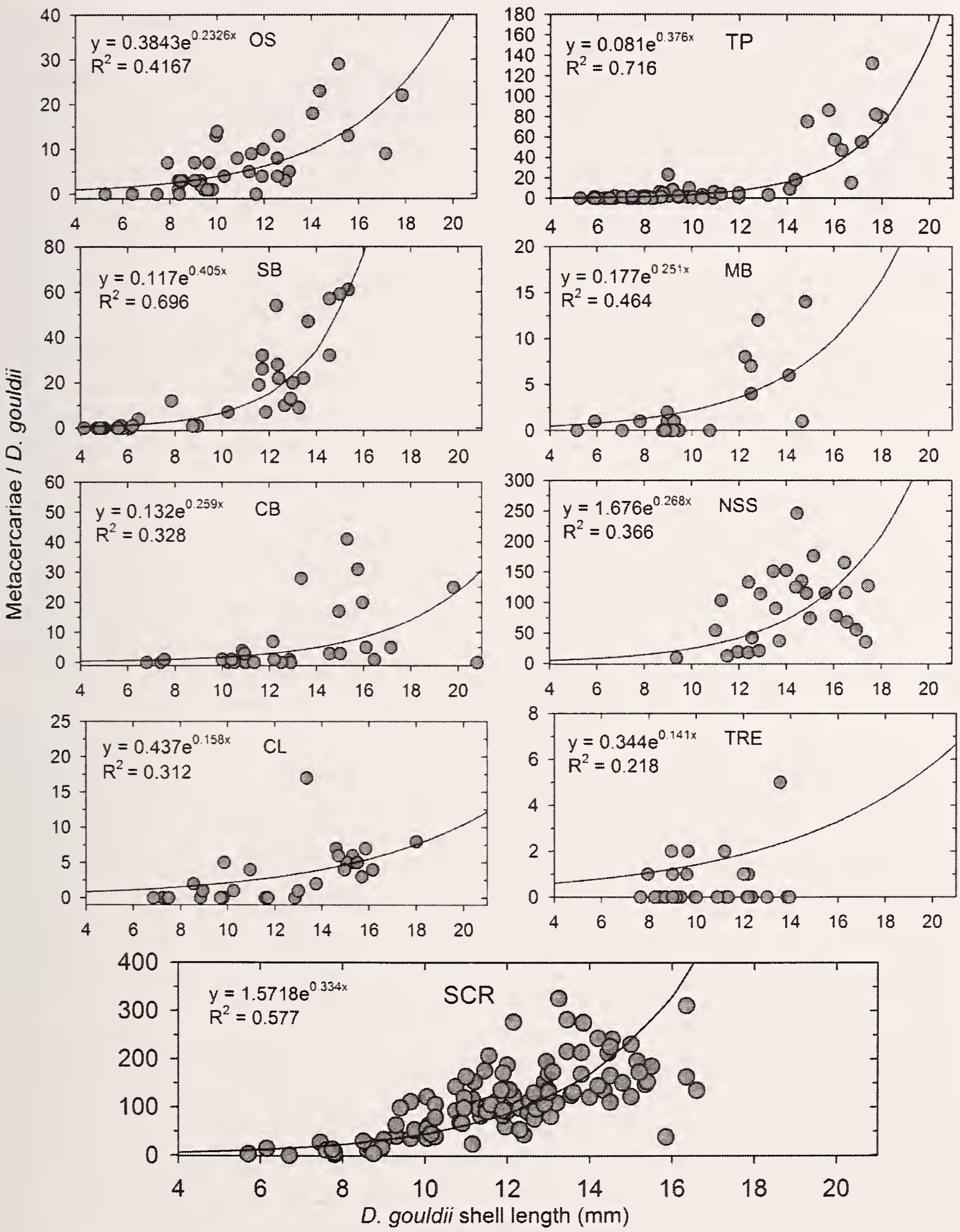

Fig. 4. Lengths of individual *D. gouldii* plotted against parasite infestation load. Each shows a positive trend between clam size and number of parasites, though the slopes of the trend lines vary by location. Displayed exponential trend lines were calculated only for parasitized individuals. Note that y-axis scaling differs by location.

average, the difference was not significant ($\alpha = 0.05$), and the clams with the two highest metacercaria counts (412 and 1221) were female, with lengths of 14.25mm and 19.25mm respectively.

The density of *D. gouldii* averaged over all locations was 5.24 clams/m^2, with a maximum location average density of 22.67 clams/m^2 and a minimum of 0 clams/m^2

Table 2. This table shows data from the Scripps Coastal Reserve from six collection dates between 1/28/2009 and 5/6/2009.

Collection Date	Mean Metacercariae	Mean Valve Length (mm)	n
1/28/2009	85	10.61	8
2/2/2009	105.0	10.28	22
2/24/2009	88.5	11.06	21
4/2/2009	123.1	12.59	20
5/6/2009	158.0	13.68	31
Total	111.7	11.83	109

(Table 1). The average densities at low (−0.3 MLLW) (7 sites), mid (0.0 MLLW) (8 sites), and high (+0.3 MLLW) (4 sites) tidal heights across all locations were 3.43 clams/m^2, 5.96 clams/m^2, and 12 clams/m^2, respectively. Clam densities at low, mid, and high tide heights ranged from 0 to 18, 0 to 16, and 0 to 34 clams/m^2, respectively. At the Oceanside location, the sixth quadrat was placed on a patch with three clams already visible, yielding a density count of 12 clams/m^2. When average density is plotted against average numbers of metacercariae found at each location, no clear pattern emerges. *Donax gouldii* were collected five times from Scripps Coastal Reserve between January 28 and May 6, 2009. The mean number of metacercariae per clam ranged from 85 to 158 on collection dates January 28 and May 6, respectively, while the mean valve length ranged from 10.61 to 13.68 for collections on January 28 and May 6, respectively (Table 2).

Discussion

As hypothesized, a positive relationship was found between the size of *D. gouldii* and the intensity of *P. donacis* metacercarial infestation at each of the nine study sites. These results were consistent with other mollusk parasitism studies (*e.g.*, Sorensen and Minchella 2001). When the data were pooled from all locations, a generally positive relationship exists; however, the mean number of metacercariae per clam and the relationship between *D. gouldii* length and number of parasites were found to change spatially. SCR had the highest mean number of metacercarial cysts per clam; the adjacent sites, TP and MB, each had relatively low averages. Similarly, NSS, with a very high mean, was next to TRE, the location with the lowest average. When infested individuals were analyzed, SB and TP showed the strongest relationships between size and infestation intensity, with exponential regressions resulting in R^2 values of 0.696 and 0.716, respectively, while TRE had the lowest R^2, at 0.218. These data showed that while the number of *P. donacis* metacercariae increases with the size of *D. gouldii* in general, spatial variability existed in the relationship on a kilometer scale; though *D. gouldii* were broadcast spawners with open populations, parasite loads must be analyzed by location in Southern California clams. There was not a clear latitudinal gradient in trematode infestation rate, nor does there appear to be a relationship between infestation rates at adjacent sites. The factors influencing this spatial variability remain unclear at this time.

It was possible that there was a parasitism threshold in *D. gouldii* before which the trematodes were unable to infest their clam hosts. Leung and Poulin (2008) found a threshold in the rate of parasitism of *Macomona liliana*, a marine bivalve, in which the rate of parasite gain was very low until individuals reached approximately 30mm, after which it increased exponentially, likely due to increased siphon size in larger individuals and a corresponding increase in water filtration rate. *Postmonorchis donacis* could be similarly limited by host size in the bean clam. Small *D. gouldii* were also young, so their lack of trematodes may be a function of lower exposure time.

In this study, three *D. gouldii* were found with severe gonadal sporocyst infestations. These data suggested that *P. donacis* can use *D. gouldii* as both a primary and a secondary intermediate host and support a two-host life history model for *P. donacis*. While the prevalence of such dual use was unknown, this study observed a 0.78% occurrence. Interestingly, the three clams found with sporocyst infestations came from the two locations with the highest average numbers of metacercariae per clam, suggesting a relationship between overall metacercarial density and the presence of *D. gouldii* with sporocyst infestations. Metacercariae were found in the clams' siphons, among the gills, and along the mantle edges; the highest concentrations were located in the siphons, and this likely plays a role in the transmission of the parasite. For example, *P. donacis* most likely infects *Menticirrhus undulatus*, the California corbina, when the fish consume exposed *D. gouldii* siphons (Love 1991).

The hypothesis that female *D. gouldii* would have more *P. donacis* metacercariae was not supported by these data. This hypothesis was based on an *a priori* assumption that females would be larger than males and therefore would have more metacercariae because of the positive relationship between size and infestation intensity. These data show that there was no significant difference between the sizes of male and female *D. gouldii* and presumably no difference in time exposed to parasites.

Time constraints on this study limited the amount of data collected and parameters examined. Further studies could investigate the parasite loads across broader scales. We examined temporal variability at SCR, but were unable to distinguish temporal effects from clam growth; a longer time scale would enable the study of *D. gouldii* at different times of the year, and parasitism rate may vary with season or water temperature. Temporal variability in trematode infestation rate could be examined at a site with a low average infestation load, such as TRE, in order to better detect changes in metacercarial density. Although *D. gouldii* density was measured in this study, greater replication would allow for a more rigorous examination of density dependence. *Donax gouldii* could be collected at more locations to provide both a larger data set and a finer spatial scale across which to examine the variability evident in the population. As with studies examining the effects of heavy metals on cercarial swimming ability (*e.g.*, Cross *et al.* 2001), data on the pollutants in nearshore water could be used to examine the effects of anthropogenic stressors on the parasitism of *D. gouldii* by *P. donacis*. The trematode, a platyhelminthe parasite that usually infects mollusks, such as snails and bivalves, and vertebrates, such as fish and birds, has dynamic and complex interactions with the environment because it uses multiple hosts. Additional research should be done to understand more fully the factors determining the infestation rates of *P. donacis* in *D. gouldii*.

This study examined the factors influencing the rate and intensity of trematode infestation in *D. gouldii*, including clam valve length, location, and density, and concluded that a positive relationship exists between clam size and parasite load and that temporal and spatial variability were evident in this relationship. Parasitism, while ubiquitous in the living world, is an understudied facet of most communities and can provide important insights into the ecologies of many organisms.

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